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Low prevalence of zoonotic multidrug resistant bacteria in veterinarians in a country with prudent use of antimicrobials in animals

LA-MRSA and ESBL-PE in Finnish veterinarians

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Summary

The occurrence of multidrug resistant, zoonotic bacteria in animals has been increasing worldwide. Working in close contact with livestock increases the risk of carriage of these bacteria. We investigated the occurrence of extended-spectrum beta-lactamase (ESBL) and plasmidic AmpC beta-lactamase producing *Enterobacteriaceae* (ESBL/pAmpC-PE) and livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) in Finnish veterinarians (n=320). In addition to microbiological samples, background information was collected. Bacterial whole genome sequencing was performed to deduce sequence types (STs), *spa* types, and resistance genes of the isolates. In total, 3.0% (9/297) of the veterinarians carried ESBL producing *Escherichia coli*, with one ESBL producing *E. coli* isolate producing also AmpC. Seven different STs, sequences of several different plasmid groups as well as several different $bla_{\text{ESBL/pAmpC}}$ genes existed in different combinations. No carbapenemase or colistin resistance genes were detected. MRSA was detected in 0.3% (1/320) of the samples. The strain belonged to LA-MRSA clonal complex (CC)398 (ST398, *spa* type 011, lacking Panton-Valentine leukocidin genes). In conclusion, this study shows low carriage of multidrug resistant zoonotic bacteria in Finnish veterinarians. However, finding LA-MRSA for the first time in a sample from a veterinarian in a country with prudent use of animal antimicrobials and regarding the recent rise of LA-MRSA on Finnish pig farms, a strong recommendation to protect people working in close contact with animals carrying LA-MRSA

CC398 is given. Further studies are needed to explain why the prevalence of LA-MRSA in veterinarians is lower in Finland than in other European countries.

Keywords

beta-Lactamases, *Escherichia coli*, Methicillin-Resistant *Staphylococcus aureus*, Veterinarians, Whole Genome Sequencing

Impacts

- Occurrence of multiresistant ESBL/pAmpC producing *Enterobacteriaceae* and LA-MRSA CC398 was studied in veterinarians in Finland.
- First study on ESBL/pAmpC producing *Enterobacteriaceae* in veterinarians shows low occurrence (3%) but whole-genome sequencing revealed high diversity of resistance genes indicating multiple sources.
- This is the first time LA-MRSA CC398 is found from a veterinarian in Finland and regarding the recent rise of LA-MRSA on Finnish pig farms, a strong recommendation to protect people working in close contact with LA-MRSA positive animals is given.

Introduction

Antimicrobial resistance is a global phenomenon increasing rapidly mainly due to the overuse and misuse of antibiotics in both humans and animals. Highly resistant zoonotic bacteria have emerged in animals, and the World Health Organization has listed several zoonotic bacteria on its global priority list of antibiotic resistant bacteria (WHO, 2017). Between animals and humans, multiresistant bacteria are transmitted through direct contact, or via food or the environment (EFSA & ECDC, 2018).

Even though evidence is partly contradictory, some studies have reported higher prevalence rates of extended-spectrum beta-lactamase and plasmidic AmpC beta-lactamase producing

Enterobacteriaceae (ESBL/pAmpC-PE*)* in people working with farm animals than in the general population (de Been et al., 2014; Dohmen et al., 2015; Huijbers et al., 2014). This indicates that animals could act as a source for human carriage. Determining the source of transmission is difficult as resistance may spread either vertically through bacterial clones (Huijbers et al., 2014) or horizontally via gene transfer (van Duijkeren et al., 2017). Dissemination of resistance genes occurs effectively via extrachromosomal plasmids belonging to several incompatibility (Inc) groups (Carattoli, 2009). Similar *Escherichia coli* sequence types (STs), ESBL/AmpC genes and plasmids have been found in farmers and animals (Huijbers et al., 2014). Although there are studies on different professions working with animals, veterinarians have not been studied for ESBL/pAmpC-PE carriage. In Finland, the proportion of resistant strains in humans is increasing (Jaakola et al., 2017) and ESBL/pAmpC-PE are found both in production and companion animals but still at a low level (EFSA & ECDC, 2018; Päivärinta, Pohjola, Fredriksson-Ahomaa, & Heikinheimo, 2016; University of Helsinki, 2017).

In Europe, livestock-associated methicillin-resistant *Staphylococcus aureus* clonal complex (CC)398 (LA-MRSA) is found in several animal species (Cuny et al., 2010) and has become an important source of human infection (Larsen et al., 2017). In many countries, it is highly prevalent in pigs (Danish Veterinary and Food Administration, 2017; Dierikx et al., 2016). People working with livestock are at increased risk of being colonized with LA-MRSA compared with the rest of the population (Lewis et al., 2008; Van Den Broek et al., 2009). Prevalence of LA-MRSA carriage among veterinarians in different European countries has ranged from 1.4% up to as high as 44% (Garcia-Graells et al., 2012; Verkade et al., 2013). Human carriers may transfer LA-MRSA strains to their household members, healthcare facilities and to animals (Grøntvedt et al., 2016; van Cleef et al., 2015). LA-MRSA strains from pigs are usually less virulent than hospital-associated and community-acquired strains, partly due to the fact that they mostly lack the Panton-Valentine leukocidin (PVL) encoding genes and genes of the human immune evasion cluster (Cuny,

Abdelbary, Layer, Werner, & Witte, 2015). The majority of LA-MRSA strains are tetracycline and zinc resistant (Cavaco, Hasman, & Aerestrup, 2011). There are indications that wearing a face mask or dust mask may protect pig farmers and their household members from persistent MRSA carriage and pig farm visitors from contamination with MRSA during short stays (Angen et al., 2019; van Cleef et al., 2015; van Cleef et al., 2014).

In Finland, prevalence of MRSA in human infections has been low, and less than 5% of invasive *S. aureus* strains have been methicillin-resistant (Jaakola et al., 2017). In contrast, the occurrence of LA-MRSA has increased rapidly in pigs, reaching 77% slaughterhouse samples in 2016–2017 (Finresvet 2016–2017, 2018). LA-MRSA CC398 has become more common also in human infections (National Institute for Health and Welfare, 2018), indicating a possible livestock-related transmission route.

The increase of resistant microbes in production animals might serve as a transmission route of these bacteria to the general population in a low human-prevalence setting. Therefore, we studied the occurrence of ESBL/pAmpC-PE and LA-MRSA CC398 in Finnish veterinarians. Background information was collected to assess possible occupational transmission routes, exposure to general risk factors such as travel, antimicrobial therapy, and hospitalisation as well as use of protective equipment. Veterinarians are widely exposed to healthy and infected animals and could be the first ones to show increased carriage rate. Furthermore, self-contamination during routine care occurs frequently among personnel in human hospitals (Krein et al., 2018), and veterinarians are working in conditions where it could be even more common. The chosen whole genome sequencing approach enabled comparison of animal and human strains on the genomic level.

Materials and methods

Study population

In December 2016, veterinarians participating in the Annual Veterinary Congress in Helsinki (Finland) were invited to participate in the study. The study was approved by the Coordinating Ethics Committee of the Hospital District of Helsinki and Uusimaa (HUS/1446/2016).

Sample collection and Web form

Nasal and oropharyngeal swabs were taken with two separate swabs (M40 Transystem Amies Agar Gel, Copan Diagnostics, Brescia, Italy) on-site. In addition, participants were given a transport swab for rectal self-sampling and asked to fill in a Web form concerning occupational and recreational risk factors as well as protective measures.

Isolation of *Enterobacteriaceae*

Rectal samples were kept at room temperature and transported to the laboratory within 24 hours of sampling. Selective isolation of ESBL, AmpC and carbapenemase producing *E. coli* and *Klebsiella pneumoniae* was done following the protocol of the EU Reference Laboratory for Antimicrobial Resistance (EUCAST) (2015). A quality control procedure to validate the selective MacConkey plates was performed using negative control strain *E. coli* ATCC 25922 and positive control strains *E. coli* CTX-M-9 (ESBL producer), *E. coli* CMY-2 (AmpC producer) and *E. coli* OXA-48 (carbapenemase producer). Single colonies morphologically typical of *E. coli* or *K. pneumoniae* were selected, recultivated onto CHROMagar Orientation plates with 1 mg/ml cefotaxime and incubated for 20–24 hours at +37°C. Single colonies of *E. coli* (light pink) or *K. pneumoniae* (metallic blue) or colonies of mixed morphology of light pink and metallic blue colour were further cultivated in Columbia bovine blood agar (Columbia blood agar base, Oxoid, Basingstoke, UK) and incubated for 20–24 hours at +37°C.

Species were identified from colony growth on blood agar based on gram staining, an oxidase test and the API 20E test (bioMérieux, Marcy-l'Étoile, France). Strains identified as *E. coli* or *K. pneumoniae* were selected for antimicrobial susceptibility testing.

Isolation of *Staphylococcus aureus*

The nose and oropharyngeal samples were transported to the laboratory at room temperature, pooled and processed within 14 hours of sampling. The samples were incubated in 5 ml Müller-Hinton broth (Oxoid, Basingstoke, UK) with 6.5% NaCl at 37°C overnight. One loopful (10 µl) was cultivated on CHROMagar MRSA plates (CHROMagar Microbiology, Paris, France) and incubated at 37°C for 24 hours. One typical colony from each plate was subcultured onto bovine blood agar plates (Columbia blood agar base, Oxoid, Basingstoke, UK) and incubated at 37°C overnight. Gram staining, coagulase testing (BD BBL Coagulase Plasmas, BD, Franklin Lakes, NJ, USA) and API Staph (BioMérieux, Marcy-l'Étoile, France) were performed to identify *S. aureus* isolates.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing of *E. coli* / *K. pneumoniae* strains was performed using the disc diffusion method of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) standard (2013; 2015). Antimicrobial discs were chosen according to EU legislation (Decision 2013/652/EU) and European Food Safety Authority (EFSA) recommendations (EFSA Panel on Biological Hazards (BIOHAZ), 2011).

Quality control was performed as described for the MacConkey plates. Inhibition zone diameters were measured following EUCAST standard guidelines (2013) and the results interpreted according to EUCAST epidemiological cut-off values (ECOFFs). The double disc synergy test was interpreted as positive if the difference between inhibition zones was ≥ 5 mm.

Isolates resistant to cefotaxime and/or ceftazidime and positive for the double disc synergy test were identified as ESBL producers. Isolates resistant to cefotaxime and/or ceftazidime and cefoxitin, but susceptible to cefepime, were identified as AmpC producers. Isolates resistant to cefotaxime and/or ceftazidime, cefoxitin and cefepime and positive for the double disc synergy test were identified as

combined ESBL and AmpC producers. Isolates resistant to cefotaxime and/or ceftazidime and meropenem were identified as carbapenemase producers.

For *S. aureus* strains, antimicrobial susceptibility was tested with the disc diffusion method using 30 µg cefoxitin discs (Rosco Diagnostica, Taastrup, Denmark). The results were interpreted according to EUCAST ECOFFs. MRSA strains AF 1214-14 MRSA and AF 1582-14 MRSA from the National Institute for Health and Welfare were used as positive reference strains and ATCC 8095 *S. aureus* and ATCC 25178 *S. aureus* as negative controls.

DNA extraction and sequencing

DNA of *E. coli* and *S. aureus* isolates was extracted and whole genome sequencing was performed on the Illumina HiSeq 4000 platform as paired end with read length of 2 × 100 bp and 100× coverage (CeGaT, Center for Genomics and Transcriptomics, Tübingen, Germany). The quality of DNA pair end read FastQ files was analysed with FastQC (Version 0.10.5-cegat; Andrews, Simon).

Detecting antimicrobial resistance and virulence genes in bacterial whole genome sequence

For ESBL, AmpC or carbapenemase producing *E. coli* and *K. pneumoniae* strains, genome assembly was performed with the PATRIC 3.3.18 Assembly Service using SPAdes. SPAdes contigs were run through the Center for Genomic Epidemiology (CGE, Technical University of Denmark, Denmark) databases. Resistance genes were searched using CGE ResFinder 2.1 (Zankari et al., 2012). Plasmid sequences of different incompatibility groups were identified by CGE PlasmidFinder 1.3's (Carattoli et al., 2014) *Enterobacteriaceae* database. Species identification was confirmed by CGE SpeciesFinder 1.2 (Larsen et al., 2014). Multilocus sequence typing was performed by MLST 1.0 (Larsen et al., 2012) with *E. coli* #1 (Wirth et al., 2006) as a reference strain.

For MRSA strains, raw reads were entered into the CGE databases MLST 1.0 (Larsen et al., 2012), spaTyper 1.0 (Bartels et al., 2014), ResFinder 2.1 (Zankari et al., 2012) and VirulenceFinder 1.5

(Joensen et al., 2014), which performed assembly before analysis for multilocus sequence type, *spa* type, resistance genes and virulence genes.

Calculation of confidence intervals

Confidence intervals (CIs) for the prevalence of ESBL/AmpC producing *E. coli* and MRSA in Finnish veterinarians were calculated using the Proportion calculator in www.openepi.com.

Results

Study population

A total of 320 veterinarians and students with the right to work as a veterinarian (after 5th year of studies) participated in the study by giving a nose and throat swab. The Web form on occupational and recreational risk factors and protective measures was filled in by 262 participants (Table 1). A rectal sample was obtained from 296 participants. All samples were given and the questionnaire filled in by 251 participants.

Prevalence of ESBL/pAmpC producing *Enterobacteriaceae*

Nine (9/296, 3%, CI 1.6–5.7%) veterinarians carried ESBL/pAmpC producing *E. coli*. ESBL/pAmpC producing *K. pneumoniae* was not detected. Initially, ten isolates were identified as *E. coli* by API 20 but one proved susceptible to 3rd generation cephalosporins in susceptibility testing. The other nine isolates were ESBL producers, and one was an AmpC producer as well (Table 2).

Presence of bla_{ESBL} and bla_{pAmpC} genes in *Escherichia coli* isolates

The ESBL genes detected through whole genome sequencing were $bla_{\text{CTX-M-15}}$ (3/9), $bla_{\text{CTX-M-14}}$ (2/9), $bla_{\text{CTX-M-1}}$ (2/9), $bla_{\text{CTX-M-27}}$ (1/9) and $bla_{\text{SHV-12}}$ (1/9). The only bla_{pAmpC} detected was $bla_{\text{CMY-76}}$ carried by an *E. coli* which also carried $bla_{\text{CTX-M-27}}$ (Table 3).

Multilocus sequence types and presence of plasmids in *Escherichia coli* isolates

STs of ESBL/pAmpC producing *E. coli* found were ST10 (1/9), ST80 (1/9), ST131 (3/9), ST450 (1/9), ST648 (1/9), ST963 (1/9) and ST1431 (1/9). Plasmids carried by the *E. coli* included several incompatibility groups. The most common plasmid was IncFII (9/9), carried by all the ESBL/AmpC producing *E. coli*. Other plasmids detected were IncFI, IncI1, IncR, IncY, IncX and IncB/O/K/Z (Table 3).

Resistance genes detected

The majority (6/9, 67%) of the ESBL/AmpC producing *E. coli* were multiresistant (resistant to at least three different groups of antimicrobials), with resistance to sulphonamides (9/9), aminoglycosides (6/9) and tetracyclines (6/9) being most prevalent (Table 3). Carbapenem or colistin resistance genes were not detected.

Prevalence of MRSA

One veterinarian was positive for MRSA carriage (1/320, 0.3% (CI 0.06–1.7%)). Phenotypically the strain was coagulase positive and resistant to cefoxitin. The multilocus ST of the strain was ST398 and *spa* type t011.

On the genomic level, the strain harboured the *bla*Z and *mec*A genes conveying resistance to beta-lactams as well as the *spc*, *nor*A, *lnu*(B), *tet*(K), *tet*(M) and *dfr*G genes associated with resistance to aminoglycosides, fluoroquinolones, lincosamides, tetracyclines (two genes) and trimethoprim, respectively. The results of the analysis for virulence genes are provided in Table 4.

Risk factors and use of protective equipment

Of all veterinarians completing the web form (n=262), 87.8% (230/262) had travelled abroad during the previous 12 months (Table 1) including travel to ESBL risk regions, with 13.7% (36/262) travelling to Asia, 1.5% (4/262) to Africa and 1.5% (4/262) to South America. Oral

treatment with antibiotics during the last 6 months was received by 34.9% (87/249) of veterinarians. Of all respondents, 6.9% (18/262) shared their households with other healthcare professionals, and 12.2% (32/262) had received hospital treatment at home and 0.8% (2/262) abroad during the previous year.

Altogether 93.5% (245/262) of participants had work-related animal contact, including contact with live animals, carcasses and samples of animal origin. Among clinicians, companion animal practice was most common, with 59.2% (155/262) treating companion animals at least weekly and 70.2% (184/262) treating companion animals less frequently. Production animal practice was most frequently cattle practice, 27.1% (71/262) having contact weekly and 43.1% (113/262) more seldom. As many as 98.1% (257/262) of veterinarians had leisure time animal contact, with dogs, cats and horses being the most prevalent contact animals. The use of a dust mask (Fig. 1) was most common in pig practice and poultry practice with 13.0% (12/93) and 12.6% (6/48) using a dust mask often or always, respectively.

Discussion

The present study shows a low carriage rate of multidrug resistant zoonotic bacteria in Finnish veterinarians, although resistance among production animals has rapidly increased during recent years. Despite frequent animal contact during work and leisure time and exposure to multiple other risk factors for multidrug resistant bacteria, the occurrence of both ESBL/pAmpC-PE and MRSA in veterinarians proved low with carriage rates of 3.0% and 0.3%, respectively.

We observed that travelling abroad and into ESBL risk regions such as Asia, Africa, and Latin America was common among veterinarians (Table 1). Compared to official statistics, Finnish veterinarians are as active travellers as the general population (91% travelled abroad in 2016) (Official Statistics of Finland, 2017). Oral treatment with antibiotics during the last 6 months was

also close to the number in the general population (Social Insurance Institution of Finland Kela, 2017).

Veterinarians from many fields of work participated in our study, including clinicians working in large animal and/or companion animal practice, government officers, university lecturers and researchers and meat-inspection veterinarians, among others. Retired veterinarians were eligible as well. Altogether 93.5% of participants had work-related animal contact, including contact with live animals, carcasses and samples of animal origin. Among clinicians, companion animal practice was most common. Production animal practice was most frequently cattle practice. However, our questions did not address seasonal or other variation in frequency of contact. Neither did we distinguish between conventional and alternative farming in the questions. Nearly all veterinarians had leisure time animal contact, with dogs, cats and horses being the most prevalent contact animals.

Our hypothesis was that as veterinarians work in close contact with animals and/or carcasses of several species and are exposed to infectious material, faeces and secretions on a regular basis, they face an additional ESBL/pAmpC load from animals in addition to the general risk factors such as travel, antibiotic treatment and hospitalisation. The strains found in this study are diverse: the genomic analysis of ESBL/pAmpC *E. coli* showed multiple different STs, $bla_{\text{ESBL/AmpC}}$ genes and plasmid families, indicating that multiple sources of transmission may exist. The source of the strains cannot be determined conclusively despite genome-based analysis of the bacteria and their plasmids. Most of the strains shared the same features as those typically found in humans in previous studies. Three veterinarians carried ESBL *E. coli* belonging to ST131 and harbouring $bla_{\text{CTX-M-15}}$, $bla_{\text{CTX-M-14}}$ or $bla_{\text{CTX-M-27}}$. The most prevalent ESBL genes in humans globally are $bla_{\text{CTX-M-15}}$ and $bla_{\text{CTX-M-14}}$, but the importance of $bla_{\text{CTX-M-27}}$ is growing (Bevan, Jones, & Hawkey, 2017). These genes are often associated with ST131. While $bla_{\text{CTX-M-15}}$ is mainly carried by plasmid family IncF as is $bla_{\text{CTX-M-27}}$ in humans, $bla_{\text{CTX-M-14}}$ is carried by several plasmid types. Two

veterinarians in this study carried strains harbouring *bla*$_{\text{CTX-M-1}}$ with IncI1 plasmids. Several studies have found such strains in food producing animals (Börjesson et al., 2016; de Been et al., 2014; Päivärinta et al., 2016). However, a strict division into human or animal strains based on resistance genes and plasmid types is not possible (Day et al., 2016). Therefore, this only allows the conclusion that transmission from animals cannot be ruled out, but most of the veterinarians carried other enzyme types than those previously found in Finnish production animals (Päivärinta et al., 2016; Pohjola et al., 2016). According to the background information received from 8/9 carriers, all worked in clinical practice, but no trend was visible in species treated. However, none of the eight carriers worked in poultry practice but two carriers reported infrequent contact to poultry during leisure time. All eight carriers had travelled abroad during the previous 12 months but not to Asia, Africa or Latin America. As many as 67% of the ESBL/pAmpC producing *E. coli* strains found in this study were genotypically multiresistant.

According to a recently published study, the prevalence of asymptomatic carriage of ESBL/AmpC producing *E. coli* and *K. pneumoniae* in Finland in 2016 was 6.8% (Rintala et al., 2018). Thus, our results indicate that working as a veterinarian does not increase carriage rates of ESBL/pAmpC-PE. Numbers from other Nordic countries with low occurrence vary from 3.2% to 4.9% (Egervärn et al., 2014; Ulstad et al., 2016). Our findings are in line with results of a study conducted in Dutch slaughterhouses which found an overall prevalence equalling that of the general population (Dohmen et al., 2017). In the Dutch study, however, prevalence varied according to the intensity of animal exposure. Differences in intensity of animal exposure might explain why farmers have shown higher prevalence of ESBL/pAmpC-PE (Dohmen et al., 2015; Huijbers et al., 2014), but this did not hold true for veterinarians in this study. Probably a farmer has more intense contact with the animals and contaminated farm environment than a veterinarian visiting the farm for a limited period of time. In addition, prevalence of ESBL/pAmpC-PE in production animals is lower in Finland than in many other European countries (EFSA and ECDC, 2018; Päivärinta et al., 2016). It

is highest in broiler chickens (8.1–14.7%). Veterinarians might also protect themselves better from faecal–oral transmission by hand hygiene practices and protective equipment than farmers.

Fortunately, no carbapenem or colistin resistance genes were found in this study. Two carbapenemase positive *E. coli* were found in two dogs in 2015 (Grönthal et al., 2018), and recently on two occasions colistin resistant strains have been found in street dogs imported from Russia (Finnish Food Safety Authority, 2017; University of Helsinki, 2018). As this study shows how frequent companion animal practice is among veterinarians, the situation should be closely monitored as infection pressure might grow rapidly due to dog imports.

Also, MRSA prevalence in this study proved low: 0.3% of the veterinarians carried MRSA. The strain belonged to the livestock-associated ST398. To our knowledge, this is the first report of LA-MRSA in Finnish veterinarians. The resistance genotype including tetracycline resistance genes *tet*(K) and *tet*(M) and *spa* type t011, and lack of PVL encoding genes of the strain found, imply that it is genetically related to livestock-associated strains. The veterinarian in question reported pig contact in the questionnaire. However, the MRSA status of the farms visited is not known. Previous studies have shown transmission from pigs to humans (Lewis et al., 2008; Van Den Broek et al., 2009), and studies also show transmission from pig farmers and veterinarians to their household members (van Cleef et al., 2015; Walter et al., 2017).

MRSA prevalence in Finnish veterinarians has been studied before in 2009 (Salmenlinna, Vainio et al. 2010). It was equally low (< 1%) but no MRSA CC398 was detected. In the targeted MRSA surveillance conducted in Finnish healthcare facilities, the proportion of humans colonized or infected with LA-MRSA has risen and was 5.0% in 2018 (National Institute for Health and Welfare, 2019). As we did not study the pig farms visited by the MRSA carrier, we cannot prove that the veterinarian in question acquired the strain from Finnish pigs. However, keeping in mind the evidence on high risks for LA-MRSA acquisition in frequent pig contact, we recommend measures to protect people working in close contact with pigs, both at farms and slaughterhouses.

The Finnish Food Safety Authority (2015) has issued a guideline to prevent spread of LA-MRSA in and between pig farms and thus diminish the risk of transmission to humans. Even more emphasis should be put on the public health aspect, to keep LA-MRSA from entering the community and to avoid the spread of LA-MRSA to Finnish hospitals. Several studies indicate that the use of a face or dust mask diminishes the risk of long-term colonization or short-term contamination of humans with LA-MRSA. However, the use of a mask is not mentioned in the guidelines of the Finnish Food Safety Authority (2015). In the web form, 59.1 % of the participants involved in pig practice reported that they never used a dust mask in pig practice. Only 13% reported using a dust mask often or always. The MRSA carrier belonged to this minority.

In addition to pigs, also contact with other animals has to be considered a possible risk factor for LA-MRSA carriage. In Finland (Salmenlinna, Lyytikäinen, Vainio et al., 2010) as well as in other countries worldwide, MRSA CC398 has been reported in horses (Cuny & Witte, 2017), which might make veterinarians working with horses another risk group. From our study population, 43.9% reported work-related contact with horses, and 62.2% had horse contact during leisure time. Nevertheless, this did not increase the occurrence of MRSA CC398 in our material. The previously reported cases were part of an epidemic in an equine hospital, and it is possible that MRSA is not widespread in Finnish horses. However, now that LA-MRSA has spread in the pig population, it is important to prevent it from spreading to other production and companion animals. Denmark already reports spillover to the mink population most likely via contaminated feed (Hansen et al., 2017).

The present study shows a low carriage rate of multidrug resistant zoonotic bacteria in Finnish veterinarians. Despite frequent occupational animal contact, ESBL/pAmpC-PE prevalence was in line with that in the general population and LA-MRSA CC398 was a rare finding. However, finding LA-MRSA CC398 for the first time from a veterinarian in Finland and considering the recent rise of

LA-MRSA prevalence in Finnish pigs, we strongly recommend measures to protect people working in close contact with animals carrying LA-MRSA CC398.

It is interesting that although LA-MRSA is frequently found in Finnish pigs, Finnish veterinarians show lower LA-MRSA carriage compared to their colleagues in other European countries. Examples from Finland and Norway show that prudent use of antimicrobials in animals does not seem to prevent the spread of LA-MRSA in animals and further spread to humans working in close contact with these animals (Grøntvedt et al., 2016). Thus, the explanation for low LA-MRSA carriage among Finnish veterinarians must be sought elsewhere. Lower pig density on farms, longer distances between farms and hygiene practices of veterinarians could serve as possible explanations and deserve further studies. The proportion of veterinarians using a dust mask on a regular basis on pig farms seems too low to explain the low prevalence. However, it would be interesting to see studies from other countries in comparison. In any case, it should be kept in mind that in Finland LA-MRSA occurrence in pigs has only recently increased, and therefore there is a possibility that without further action, prevalence among veterinarians will increase in the near future.

Conflicts of Interest

None to declare.

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Table 1. Background characteristics, risk factors, and carriage of MRSA and ESBL of veterinarians participating in the study and submitting the Web form (n=262).

Category	Subcategory	No. (%) of participants	No. of carriers	
			MRSA (n=1)	ESBL/pAmpC-PE (n=8)
General characteristics				
Gender	female	230 (87.8)	-	5
	male	30 (11.5)	-	3
	not disclosed	2 (0.8)	-	0
Years since graduation	0–9	112 (42.7)	-	0
	10–20	76 (29.0)	-	5
	>20	74 (28.2)	-	3
Location of veterinary education	Helsinki, Finland	236 (90.1)	-	8
	Abroad	26 (9.9)	-	0
Animal-related risk factors				
Work-related animal contact[†]	Yes	245 (93.5)	1	8
	No	17 (6.5)	0	0
Type of animal contact	Pig practice	77 (29.4)	1	2
	Cattle practice	113 (43.1)	1	4
	Poultry practice	42 (16.0)	0	0
	Fur animal practice	11 (4.2)	0	0
	Equine practice	115 (43.9)	0	3
	Companion animal practice	184 (70.2)	0	6
Type of frequent[‡] animal contact	Pig practice	17 (6.5)	0	1
	Cattle practice	71 (27.1)	0	2
	Poultry practice	7 (2.7)	0	0
	Fur animal practice	0 (0.0)	0	0
	Equine practice	41 (15.6)	0	3
	Companion animal practice	155 (59.2)	0	1

Work-related animal contact abroad	Yes	21 (8.0)	0	1
	No	241 (92.0)	0	0
Leisure time animal contact	Yes	257 (98.1)	0	8
	No	5 (1.9)	1	0
Type of leisure time animal contact[§]	Pigs	27 (10.3)	0	2
	Cattle	76 (29.0)	0	3
	Poultry	42 (16.0)	0	2
	Fur animals	2 (0.8)	0	0
	Horses	163 (62.2)	0	4
	Dogs	251 (95.8)	0	8
	Cats	219 (83.6)	0	8
Frequent[‡] leisure time animal contact	Pigs	4 (1.5)	0	1
	Cattle	10 (3.8)	0	1
	Poultry	12 (4.6)	0	0
	Fur animals	0 (0.0)	0	0
	Horses	68 (26.0)	0	2
	Dogs	179 (68.3)	0	6
	Cats	114 (43.5)	0	6
Occupation of household member	Veterinarian/ veterinary technician	27 (10.3)	0	1
	Other profession with animal contact	24 (9.2)	0	0
Travel-related risk factors				
International travel[¶]	Yes	230 (87.8)	1	8
	No	32 (12.2)	0	0
Travel regions	Nordic countries	134 (51.1)	1	5
	Baltic countries	93 (35.5)	1	3
	Central Europe	118 (45.0)	1	6
	Southern Europe	95 (36.3)	1	2
	Eastern Europe	22 (8.4)	0	0
	Asia	36 (13.7)	1	0
	North America	14 (5.3)	0	0
	Central America	4 (1.5)	0	0
	South America	4 (1.5)	0	0

	Africa	4 (1.5)	0	0
	Australia and Oceania	3 (1.1)	0	0
Healthcare-related risk factors				
Occupation of household member	Healthcare professional	18 (6.9)	0	0
Hospitalised[¶]	In Finland	32 (12.2)	0	1
	Abroad	2 (0.8)	0	0
Chronic immunocompromising disease	Yes	7 (2.7)	0	1
	No	254 (96.9)	0	8
	Not disclosed	1 (0.4)	0	0
Course of oral antibiotics	Within last month	16 (6.6) (n=241)	0	0
	Within last 6 months	87 (34.9) (n=249)	n/a	4
Verified previous colonisation/ infection with MRSA and/or ESBL producing *Enterobacteriaceae*		0 (0.0)	0	0

[†]Animal contact was defined as contact with live animals, carcasses or samples of animal origin.
[‡] At least once a week.
[§] Less than once a week.
[¶] During last 12 months.
MRSA: Methicillin-resistant *Staphylococcus aureus.*
ESBL: Extended-spectrum beta-lactamase.
ESBL/pAmp-C-PE: Extended-spectrum beta-lactamase and/or plasmidic AmpC producing *Enterobacteriaceae*
- To protect respondent confidentiality information is not published in this table.
n/a not available

Table 2. Susceptibility profiles of *Escherichia coli* strains isolated from veterinarians.[†]

Strain	CTX	CAZ	FOX	FEP	MEM	CTX-C, CAZ-C	Phenotype
EL15E	S	S	S	S	S	--	--
EL24E	R	R	S	R	S	+, +	ESBL[‡]
EL120E	R	S	S	R	S	+, −	ESBL
EL158E	R	R	S	R	S	+, +	ESBL
EL216E	R	R	S	R	S	+, +	ESBL
EL233E	R	R	S	R	S	+, +	ESBL
EL245E	R	R	S	R	S	+, +	ESBL
EL256E	R	R	S	R	S	+, +	ESBL
EL259E	R	R	S	R	S	+, +	ESBL
EL298E	R	R	R	R	S	+, +	ESBL+AmpC

[†] Inhibition zone diameters were measured following the European Committee on Antimicrobial Susceptibility Testing (EUCAST) standard guidelines (EUCAST, 2013) and results interpreted according to EUCAST epidemiological cut-off values.

[‡] extended-spectrum beta-lactamase producer

CTX: cefotaxime 5 μg CAZ: ceftazidime 10 μg FOX: cefoxitin 30 μg FEP: cefepime 30 μg MEM: meropenem 10 μg CTX-C: cefotaxime 30 μg and cefotaxime/clavulanic acid 30 μg/10 μg CAZ-C: ceftazidime 30 μg and ceftazidime/clavulanic acid 30 μg/10 μg

S: susceptible, R: resistant, -- not performed, + positive result, − negative result.

Table 3. Characteristics of extended-spectrum beta-lactamase (ESBL) producing *Escherichia coli* isolates carried by Finnish veterinarians.

Isolate	Phenotype	Sequence type	Plasmids	3rd generation cephalosporins, ESBL	3rd generation cephalosporins, AmpC	2nd generation cephalosporins	Carbapenem	Aminoglycoside	Phenicol	Fosfomycin	Fusidic acid	Glycopeptide	Fluoroquinolone	Colistin	Macrolide, lincosamide and streptogramin B	Nitroimidazole	Oxazolidinone	Rifampicin	Sulphonamide	Tetracycline	Trimethoprim
EL15E	S	1431	*IncFIB, IncFIC*	–	–	$bla_{\text{TEM-1A}}$	–	*strA* *straB* *aad5A*	–	–	–	–	–	–	*mph(A)*	–	–	–	*sul1* *sul2*	*tet(A)*	*dfrA17*
EL24E	ESBL	131	*IncFII, IncFIA, IncX4, IncFIB, Col(BS512), Col156*	$bla_{\text{CTX-M-27}}$	$bla_{\text{CMY-76}}$	–	–	*aac(6')If*	–	–	–	–	–	–	–	–	–	–	–	–	–
EL120E	ESBL	450	*IncB/O/K/Z, IncFIB, IncFII, IncFIA, Col156, Col(BS512), Col(KPHS6)*	$bla_{\text{CTX-M-14}}$	–	$bla_{\text{TEM-1B}}$	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
EL158E	ESBL	10	*IncI1, IncFIB, IncFII*	$bla_{\text{CTX-M-1}}$	–	–	–	–	–	–	–	–	–	–	–	–	–	–	*sul2*	*tet(A)*	–
EL216E	ESBL	131	*IncFII, IncFIA, IncFIB*	$bla_{\text{CTX-M-15}}$	–	–	–	*aadA2*	–	–	–	–	–	–	*mph(A)* *erm(B)*	–	–	–	*sul1*	–	*drfA12*
EL233E	ESBL	80	*IncFII, Col(BS512)*	$bla_{\text{CTX-M-14}}$	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
EL245E	ESBL	963	*IncI1, IncFII, IncFIB, IncFIB*	$bla_{\text{CTX-M-1}}$	–	–	–	–	–	–	–	–	–	–	–	–	–	–	*sul2*	*tet(A)*	–
EL256E	ESBL	131	*IncFII, IncFIB, IncY, IncB/O/K/Z*	$bla_{\text{CTX-M-15}}$	–	$bla_{\text{TEM-1A}}$	–	*straA* *straB* *aad(3)IId*	–	–	–	–	–	–	–	–	–	–	–	–	–
EL259E	ESBL	871	*IncFII, IncR, IncY, IncX1*	$bla_{\text{SHV-12}}$	–	$bla_{\text{TEM-1A}}$	–	*aadA1* *aadA2* *aad(3)IIa* *aph(3')IIa*	*floR* *cmlA1*	–	–	–	*QnrS1*	–	Inu (F)	–	–	–	*sul3*	*tet(A)*	*dfrA1*
EL298E	ESBL+ AmpC	648	*IncFIA, IncFIB, IncFII*	$bla_{\text{CTX-M-15}}$	–	$bla_{\text{OXA-1}}$	–	*aac(6')Ibcr* *aadA5*	*catB3*	–	–	–	*aac(6')Ibcr*	–	*mph(A)*	–	–	–	*sul1*	*tet(B)*	*dfrA17*

S: Susceptible to 3rd generation cephalosporins. – not detected.

Table 4. Virulence gene detection by VirulenceFinder 1.5 (Joensen et al., 2014) in livestock-associated methicillin-resistant *Staphylococcus aureus* CC398 isolated from veterinarian.

Virulence gene	+/−
hlb	+
*hlg*ABC	+
tst	−
*luk*ED	−
*luk*FS-PV	−
*et*AB	−
*edin*ABC	−
aur	+
spc ABE	−
scn	−
sak	−
ACME	−
*Se*A-E, G-O, R, U, Q	−

+ detected; − not detected

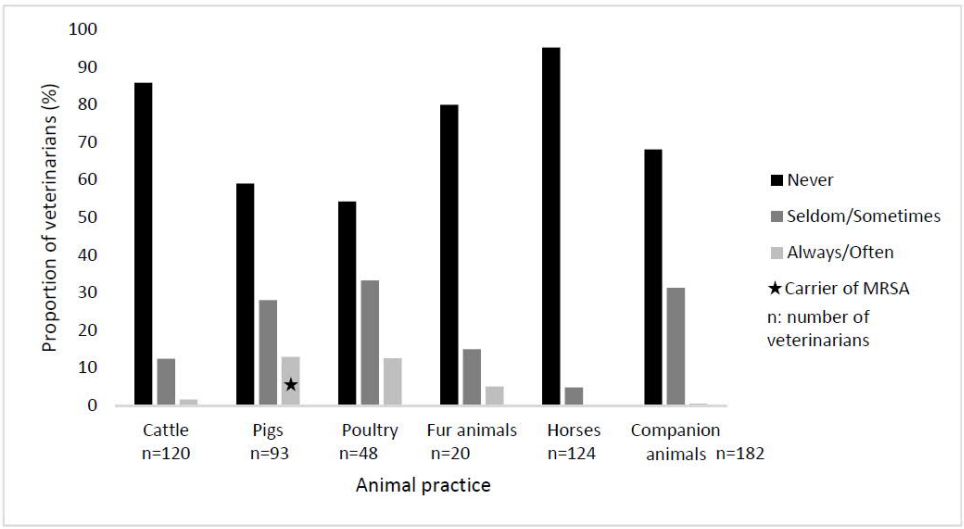

Figure 1. Use of dust masks by veterinarians in animal practice. (Surgical procedures and dental care excluded.)